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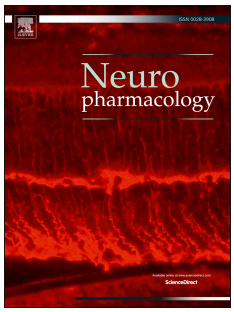

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ACCEPTED MANUSCRIPT

Agonist and antagonist bind differently to 5-HT$_{1A}$ receptors during Alzheimer's disease: a *post-mortem* study with PET radiopharmaceuticals

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Abstract

PET imaging studies using 5-HT_{1A} receptor radiotracers show a decreased density of this receptor in hippocampi of patients with Alzheimer's disease (AD) at advanced stages. However, current 5-HT_{1A} receptor radiopharmaceuticals used in neuroimaging are antagonists, thought to bind to 5-HT_{1A} receptors in different functional states (i.e., both the one which displays high affinity for agonists and is thought to mediate receptor activation, as well as the state which has low affinity for agonists). Comparing the PET imaging obtained using an agonist radiotracer, which binds selectively to functional receptors, with the PET imaging obtained using an antagonist radiotracer would therefore provide original information on 5-HT_{1A} receptor impairment during AD. Quantitative autoradiography using [^{18}F]F13640 and [^{18}F]MPPF, a 5-HT_{1A} agonist and antagonist, respectively, was measured in hippocampi of patients with AD (n=25, at different Braak stages) and control subjects (n=9). The neuronal density was measured in the same tissues by NeuN immunohistochemistry. The specific binding of both radiotracers was determined by addition of WAY-100635, a selective 5-HT_{1A} receptor antagonist. The autoradiography distribution of both 5-HT_{1A} PET radiotracers varied across hippocampus regions. The highest binding density was in the pyramidal layer of CA1. Incubation with Gpp(NH)p, a non-hydrolysable analogue of GTP, reduced significantly [^{18}F]F13640 binding in hippocampal regions, confirming its preferential interaction with G-coupled receptors, and slightly increased [^{18}F]MPPF binding. In the CA1 subfield, [^{18}F]F13640 binding was significantly decreased at Braak stages I/II (-19%), Braak stages III/IV (-23%), and Braak stages V/VI (-36%) versus control. In contrast, [^{18}F]MPPF binding was statistically reduced only at the most advanced Braak stages V/VI compared to control (-33%).

Since [^{18}F]F13640 and [^{18}F]MPPF can be used in vivo in humans, this neuropharmacological paradigm supports testing the concept of functional imaging using agonist radiopharmaceuticals in future clinical studies.

ACCEPTED MANUSCRIPT

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles, resulting from an abnormal aggregation of the amyloid beta-peptide and the hyperphosphorylated protein tau, respectively. In addition to these protein aggregates, the progressive loss of neurons and multiple neurotransmitter systems impairment represent the pathological hallmark of Alzheimer's disease (Tampellini, 2015; Raskin et al, 2015). Although the loss of cholinergic fibers seems to be closely related to cognitive impairment, perturbations of other neurotransmitter systems may be involved in the pathophysiology of the disease. There is increasing evidence that the serotonergic system is highly dysfunctional in AD, which could be related to several clinical symptoms of dementia (Ramirez et al, 2014). Many *post-mortem* studies have reported a loss of serotonin synthesizing neurons and modifications of serotonergic receptors densities in brains of AD patients (Halliday et al., 1992; Lorke et al., 2006; Salmon, 2007). In particular, several studies have shown a reduction of 5-HT_{1A} receptors levels (Bowen et al., 1989; Cross et al., 1988; Middlemiss et al., 1986). Serotonergic 5-HT_{1A} receptors are of particular interest since they are strongly expressed in the hippocampus, which is heavily involved in functions such as memory, behavior and emotions (Albert et al, 2014; Borg, 2008; Sarnyai et al., 2000). Although several 5-HT_{1A}-targeting molecules have been proposed to treat cognitive or non-cognitive symptoms in AD, results have been inconsistent and converging preclinical and clinical data are still awaited to validate this receptor as a therapeutic target in AD (Schechter et al., 2005; Sato et al., 2007; Depoortere et al., 2010). Notably, precise links between 5-HT_{1A} receptors and cognition remains to be elucidated to validate this target for treatment of cognitive deficits (Borg, 2008; Ramirez, 2014). Because the earliest severe cognitive deficit in AD concerns episodic memory linked to neurofibrillary tangles in the hippocampus (Dubois et al., 2007; Serrano-Pozo et al., 2011), several studies have focused on 5-HT_{1A} receptor status in the hippocampus of AD living patients by Positron Emission Tomography (PET) imaging. Using the radiolabeled antagonist [^{18}F]MPPF, these studies have shown a decrease of 5-HT_{1A} receptor density in the hippocampus of advanced AD patients (Kepe et al., 2006; Lanctôt et al., 2007). On the contrary, an up-regulation of 5-HT_{1A} receptors has been reported at the Mild Cognitive Impairment (MCI) stage, prodromal of AD (Truchot et al., 2007; Truchot et al., 2008). The pathophysiological significance of this phenomenon remains to be elucidated, and could reflect compensatory mechanisms occurring at the earliest stages of this neurodegenerative disease (Truchot et al., 2007; Verdurand et al., 2011).

An improved understanding of the functional role of 5-HT_{1A} receptors in AD may be gained by taking into account additional aspects of their pharmacological profile. Indeed, as G-protein-coupled receptors (GPCR), they have been shown to exist in different states (Mongeau et al., 1992; Nénonéné

et al., 1994): a high-affinity state for agonists, (effective receptor/G-protein coupling, supposedly active), and a low affinity (G-protein uncoupled, non-functional). Contrary to an agonist, an antagonist, like [^{18}F]MPPF used in previous PET studies, indiscriminately labels all 5-HT$_{1A}$ receptors, regardless of their G-protein coupling state (Gozlan et al., 1995; Burnet et al., 1997). Furthermore, growing evidence suggests that changes in receptor/G-protein coupling may be involved in neurodegenerative disorders (Thathiah and De Strooper, 2011). It has to be noted that the functional (G-protein coupling) state of receptors has not been taken into account in the interpretation of previous PET study data, particularly in AD patients. Therefore, comparing the 5-HT$_{1A}$ receptor binding detected by antagonist and agonist PET radioligands could provide additional information concerning the proportion of "functional receptors" during AD (Figure 1).

The aim of this study was to investigate the modifications of 5-HT$_{1A}$ receptor coupling in human brain during AD, using autoradiography on *post-mortem* hippocampus sections. We compared on the same patients the binding profiles of [^{18}F]F13640, a highly selective 5-HT$_{1A}$ receptors 'full agonist' currently evaluated in a first-in-man study as a PET radiopharmaceutical (Vidal et al., 2014; EU Clinical Trials Register, EudraCT number #2016-00016-15), and of [^{18}F]MPPF, a 5-HT$_{1A}$ receptors antagonist which has already been widely used in clinical PET studies (Cheng, 2005; Aznavour and Zimmer, 2007). We hypothesized that we would observe different modifications between the "total" and "coupled" 5-HT$_{1A}$ receptors states over the course of the disease, supporting the use of 5-HT$_{1A}$ receptor agonists in PET imaging of neurodegenerative disorders. As some studies interpreted 5-HT$_{1A}$ receptor changes in terms of neuronal density (Bowen et al., 1989; Kepe et al., 2006; Lanctot et al., 2007), we also investigated the number of pyramidal cells in the same brain areas by NeuN immunohistochemistry.

2. Methods

2.1. Subjects and tissue samples

Fresh frozen hippocampus sections were obtained from the MRC London Neurodegenerative Diseases Brain Bank after approval by the brain bank's scientific review committee. Brain tissue of 34 subjects were included, with 9 control subjects, 5 Braak I-II subjects, 10 Braak III-IV subjects and 10 Braak V-VI subjects (Table 1). Patient ages, *post-mortem* delay and sex ratio were not significantly different among the patient groups (all $p>0.05$). The Braak staging was based on assessment of immunohistochemically labeled hyperphosphorylated tau immunoreactive neuropil threads, according to the BrainNet Europe consortium (Alafuzoff et al., 2008). Stage 0 was characterized by a total absence of neurofibrillary pathology and was used as control group; stages I, II, and III had

changes confined to the transentorhinal and entorhinal regions, and a beginning of AT8 immunopositive neurofibrillary tangles in temporal and temporo-occipital cortices; stages IV, V, and VI had marked destruction of these regions, extending to isocortical association areas, namely the occipital cortex. Each case included 15 successive hippocampus sections of 30 µm thickness. The sections were stored at -80°C without formalin fixation for autoradiography experiments (two sections per slide).

2.2. Radiosyntheses and quality controls

On the days of autoradiography experiments, [^{18}F]MPPF and [^{18}F]F13640 were synthesized in an automated radiosynthesizer (Neptis, ORA) according to previously described radiochemical pathways (Le Bars et al., 1998; Vidal et al., 2014). Their chemical and radiochemical purities, as measured by HPLC, were higher than 98%. The specific activity at time of autoradiography was calculated at 37 GBq/µmol (1 Ci/µmol) for [^{18}F]MPPF, and 11.1 GBq/µmol (0.3 Ci/µmol) for [^{18}F]F13640.

2.3. Quantitative 5-HT$_{1A}$ receptors autoradiography

Defrosted slides were incubated for 20 min in Tris phosphate-buffered saline buffer (138 mM NaCl, 2.7 mM KCl, pH adjusted to 7.5) containing 37 kBq/mL (1 µCi/mL) of [^{18}F]MPPF or [^{18}F]F13640. For each radioligand, the specific activity was calibrated so that the concentration added in the buffer was equal to three times the Kd. Thus, the sections were incubated with either 1 nM of [^{18}F]MPPF or 3 nM of [^{18}F]F13640, with known Kd of 0.3 nM and 1 nM, respectively (Kung et al., 1996; Maurel et al, 2007). Non-specific binding was determined in duplicate serial sections co-incubated with 10 µM WAY-100635 (Sigma-Aldrich). For verification of the agonistic binding of [^{18}F]F13640, the corresponding buffer was supplemented with Gpp(NH)p (10 µM), a non-hydrolysable analogue of GTP that elicits decoupling of G-protein-coupled receptors. After incubation, the slides were dipped in cold buffer and distilled water (4°C), then dried and juxtaposed to a phosphor imaging plate for 60 min (BAS-5000, Fujifilm). All films were analyzed by a computer-assisted image analysis system (Multigauge, Fujifilm), and regions of interest (CA1, dentate gyrus) were drawn manually, according to a human brain atlas (Mai and Paxinos, 1997) and confirmed by a following Luxol-Fast Blue staining and by a NeuN immunostaining (procedure described below). Quantitation in each region of interest was performed by measuring the average optical density in the adjacent brain sections. In parallel, calibration standards were prepared from rat brain tissue homogenates, following the same procedure described in our previous *post-mortem* study (Becker et al., 2014). Briefly, rat brains were extracted, homogenized and their mass was determined after aliquoting in micro-vials before 80°C congelation. Their protein content was quantified using a chemistry analyzer (Architect Ci8200, Abbott Diagnostics). On the day of experiment, increasing activities of radioligand were mixed with

these homogenates, which were then immediately frozen and cut into 30 µm coronal section. These extemporaneous standards were juxtaposed to the same imaging plates used for the human tissue, and signal to concentration curves were generated. Non-specific binding was subtracted from the total binding to determine the specific binding, and measurements were converted into fmoles of ligand/mg of protein, according to the calibration curve obtained from the standards.

2.4. NeuN immunostaining and neuron counting

One slide per subject was processed for immunohistochemistry using a mouse monoclonal antibody directed against the neuron-specific nuclear antigen NeuN (mAB377 A60, Chemicon, 1/500). Images of the entire sections were taken using a slide scanner (Zeiss Axioscan, CIQLE microscopy platform, Lyon). The whole images were used to validate the regions of interest (ROIs) delimitation on the autoradiographs and to estimate neuronal densities (Figure 2). NeuN-positive cells were manually counted in the CA1 area using the ImageJ cell counter (Schneider et al., 2012). Each counting was done by two different experimenters who were blind for the subjects' groups. In the dentate gyrus, cells were automatically counted by the Icy software (tool "Spot detector"), by applying pre-defined counting parameters. Some subjects were excluded from this part of the study because the detected spots did not match with the NeuN-positive cells. In both regions of interest, the result was expressed in number of neurons per mm^3.

2.5. Statistical analyses

Demographics were compared among the groups using the Kruskal-Wallis non parametric test. Statistically significant variations in radioligand binding and neuronal density, for each Braak stage versus control, were measured by Mann-Whitney non parametric test. For all the analyses, the statistical significance was set at $p<0.05$ (GraphPad Prism6).

3. Results

3.1. Autoradiography

The autoradiography distribution of both 5-HT_{1A} receptors radiotracers varied across the hippocampus sub-regions (Figure 1). The highest binding density was found in the pyramidal cell layer of CA1, whereas it was moderate in the dentate gyrus and CA3, consistent with previous findings (Burnet et al., 1995; Becker et al, 2014). The addition of the 5-HT_{1A} receptors antagonist WAY-100635 at 10 µM in the buffer resulted in a 85 to 90% decrease of [^{18}F]F13640 and [^{18}F]MPPF binding, confirming their 5-HT_{1A} receptor specificity. The addition of Gpp(NH)p in the buffer led to a

70% decrease of the [^{18}F]F13640 binding. In the same conditions, a slight increase of [^{18}F]MPPF binding was measured after addition of Gpp(NH)p (+17%) (see Figure 3).

Comparison of [^{18}F]F13640 and [^{18}F]MPPF binding identified different modifications of 5-HT$_{1A}$ receptor density according to the Braak stages, and depending on the hippocampal subfield considered (Figure 4). In the CA1 subfield, [^{18}F]F13640 binding was significantly decreased at Braak stages I/II (-19%; $p<0.05$), Braak stages III/IV (-23%; $p<0.05$), and Braak stages V/VI (-36%; $p<0.01$) versus control. In contrast, [^{18}F]MPPF binding was statistically reduced only at the most advanced Braak stages V/VI compared to control (-33%; $p<0.01$). In the dentate gyrus, [^{18}F]MPPF labeling was unchanged at late stages compared to control, but significantly increased at the early stages Braak I/II (+26%; $p<0.05$). On the contrary, [^{18}F]F13640 binding was unchanged at these same stages, but strongly decreased at Braak stages V/VI (-40%; $p<0.01$).

3.2 Neuron counting

In the CA1 subfield, the neuronal density quantified by NeuN immunohistochemistry was unchanged in the AD patients versus the control group, until the latest Braak stages V/VI (-38%; $p<0.05$). In the dentate gyrus, it was unchanged at advanced Braak stages but increased in the early Braak stages I/II (+53%; $p<0.05$; see Figure 2).

4. Discussion

The neuropharmacological hypothesis of our study was that the agonist radiotracer [^{18}F]F13640, that preferentially labels G-protein-coupled 5-HT$_{1A}$ receptors, would detect a different profile of 5-HT$_{1A}$ receptors modifications during AD in comparison to the antagonist radiotracer [^{18}F]MPPF which labels the entire 5-HT$_{1A}$ receptors population. Both radiotracers are highly selective for 5-HT$_{1A}$ receptors, with a comparable nanomolar affinity (Kung et al., 1997; Colpaert et al., 2002). The agonist properties of [^{18}F]F13640 and its selectivity for G-protein-coupled functional receptors, previously described in rat brain (Vidal et al, 2014), were confirmed by the uncoupling action of Gpp(NH)p which decreased sharply the radiotracer binding in human tissues. Conversely, under similar conditions, the binding of the antagonist [^{18}F]MPPF was slightly increased in the presence of Gpp(NH)p, confirming previous studies (Lemoine et al, 2010). In a recent study, we proposed that the coupling state of 5-HT$_{1A}$ receptors was altered before the loss of receptors themselves in AD (Becker et al., 2014). However, this previous study was preliminary since the number of patients was small. Moreover, neuronal density was not determined and the agonist radiotracer we used, [^{18}F]F15599, is

not developed as a clinical research PET radiopharmaceutical, limiting the translational impact of the results.

As detailed above, the present study was carefully designed to ensure the validity of results, supported by a robust methodology. (i), for each radioligand and for each radiosynthesis, samples from all 34 patients were tested simultaneously to avoid inter-variability between radiosyntheses or between autoradiography films; (ii), the normalization of the results was improved by the use of calibration curves enabling the quantification of the binding intensity in fmoles of radioligand per gram of tissue; (iii), for each radioligand, the specific activity and the incubation concentration were calibrated so that the concentration added in the buffer was equal to three times the Kd, to ensure optimal saturating concentrations (Burnet et al., 1997); (iv), the fact that the agonist [^{18}F]F13640 binds preferentially to G-protein-coupled 'functional' 5-HT$_{1A}$ receptors was confirmed by the receptor/G-protein decoupling action of Gpp(NH)p; (v); for each patient, serial sections were used, limiting inter-individual variability; (vi), in terms of patient stratification, the Braak staging was used to sort patients by degree of Tau pathology as it has been demonstrated that there is a good correlation between hyperphosphorylated tau deposits and the cognitive status (Braak and Braak, 1991; Alafuzoff et al., 2008); (vii), the different patient groups were well-matched in terms of age and *post-mortem* delay, an important point to consider since these demographic values are known to potentially impact the G-protein coupling state of receptors (Gonzalez-Maeso et al., 2002). However, as is frequent in studies using brain bank tissues, it should be noted that we did not have access to the clinical data relative to the *pre-mortem* psychiatric status and the possible psychotropic drugs taken by each patient.

In the CA1 subfield of hippocampus, the [^{18}F]MPPF binding, reflecting the total number of 5-HT$_{1A}$ receptors, was decreased in the latest Braak stages V/VI compared to control, which is consistent with the literature. In particular, an immunohistochemical study showed very similar results, with a 5-HT$_{1A}$ receptor loss specifically observed in CA1 and only in Braak stages V/VI (Mizukami et al., 2011). Although most of the other *post-mortem* studies also showed a decrease in 5-HT$_{1A}$ receptors in AD patients' brain (Bowen et al., 1989; Francis et al., 1993; Cross et al., 1988), the neuropathological status of the patients was not systematically taken into account. Similarly, several PET imaging studies of 5-HT$_{1A}$ receptors reported a decrease of [^{18}F]MPPF binding in the hippocampus of patients with advanced AD, but results were divergent at predementia stages (Kepe et al., 2006 ; Lanctot et al., 2007 ; Truchot et al., 2007). Taken together, these results are in favor of a modest decrease of 5-HT$_{1A}$ receptors density in AD, in several areas including the hippocampus, and measurable at the advanced stages of the disease.

Interestingly, in the case of [^{18}F]F13640 binding (reflecting the functional population of 5-HT$_{1A}$ receptors), a significant decrease was detected at an earlier Braak stage, i.e. in all AD groups

including the early Braak stages I/II. These findings support the occurrence of an early uncoupling of 5-HT_{1A} receptors in the CA1 subfield, prior to the loss of receptors themselves, when neurofibrillary tangles are sparsely detectable in the hippocampus (Raskin et al, 2015). Other studies also suggested that GPCR function is altered during AD (Thathiah and De Strooper 2011), especially in the CA1 subfield (Garcia-Jimenez et al., 2002), and including/affecting 5-HT_{1A} receptors (Weinstein et al 1996). The pathophysiological meaning of this 5-HT_{1A} receptors uncoupling in CA1 remains to be investigated but we can hypothesize that it might lead to an impaired intracellular signaling prior to the apparition of clinical signs (Garcia-Jimenez et al., 2002).

Another observation we made was that, compared to other hippocampal subfields, CA1 was particularly affected by both loss of 5-HT_{1A} receptors at the latest Braak stages (detected by [^{18}F]MPPF labeling) and uncoupling of 5-HT_{1A} receptors at the early stages (detected by [^{18}F]F13640 labeling). This observation could result from the stronger density of 5-HT_{1A} receptors in CA1, making changes in this hippocampal subfield more easily detectable. Additionally, this subfield may be particularly vulnerable to AD processes (Garcia-Jimenez et al., 2002; Mizukami et al., 2011; De Flores et al., 2015).

In the dentate gyrus, the [^{18}F]F13640 signal was decreased only in the Braak V/VI group compared to the control one, and no reduction of [^{18}F]MPPF binding was observed in this area during disease progression. On the contrary, there was a significant increase of [^{18}F]MPPF signal in the Braak I/II group. This surprising result is quite similar to previous findings from in vivo PET studies which reported an increase of [^{18}F]MPPF binding in the hippocampus at an early stage of the disease in MCI patients (Truchot et al, 2007). This observation was also replicated in our laboratory in a rodent model of AD where the infusion of the amyloid beta-40 peptide in the rat hippocampus induced a transient 5-HT_{1A} receptors overexpression specific to the dentate gyrus (Verdurand et al, 2011; 2016). Such an early increase in hippocampal [^{18}F]MPPF binding may be explained by the transient hippocampal hyperactivity that has been extensively observed in MCI patients in terms of cholinergic neurotransmission (DeKosky et al, 2002), fMRI activation (Dickerson et al, 2004; 2005) or glucose metabolism (Tahmasian et al, 2015). It is mainly interpreted as a compensatory mechanism that would later be challenged by disease progression at the dementia stage. Therefore, the 5-HT_{1A} receptors transient "rebound" in the hippocampus may be another sign of this hyperfunction. Importantly, we did not observe such increase in the dentate gyrus with the agonist [^{18}F]F13640, which would mean that the overexpressed 5-HT_{1A} receptors are in a non-functional state. Therefore, the pathophysiological link between this phenomenon and possible compensatory mechanisms is unclear. Since this serotonergic hyperactivity seems specific to the dentate gyrus, it could be linked to the neurogenesis that occurs in this hippocampal sub-region (Verdurand et al., 2016). The possibility of increased hippocampal neurogenesis during AD is nonetheless still a matter of debate

(Mu and Gage, 2011; Jin et al., 2004; Donovan et al., 2006). This early serotonergic hyperactivity could also be due to an astrocytic reaction, which has been reported in pre-senile AD cases and animal models (Boekhoorn et al., 2006; Verdurand et al., 2011) and may reflect astrocyte expression of 5-HT_{1A} receptors in their inactive state (Hirst et al., 1998) (although other studies have questioned astrocytic localization of 5-HT_{1A} receptors (Kia et al., 1996)). More recently, increased hippocampal activation in MCI has also been hypothesized to be a dysfunctional condition instead of a beneficial functional compensation (Bakker et al., 2012; Tahmasian et al., 2015). Indeed, 5-HT_{1A} receptors are coupled to Gi/o proteins for signal transduction, and their activation in hippocampus results in adenylyl cyclase inhibition and GIRK activation which strongly inhibits the neuronal firing and excitability (Polter and Li, 2010). An early loss of 5-HT_{1A} receptors functionality could therefore indirectly contribute to a greater and potentially harmful hippocampal activity.

In several previous PET imaging studies, the total density of 5-HT_{1A} receptors (as measured by an antagonist radioligand) was interpreted as an indirect marker of neuronal density, therefore providing a convenient way to estimate the pyramidal cells loss in the living brain of AD patients (Kepe et al., 2006; Bowen et al 1989; Mizukami et al 2011). In our study, we quantified the pyramidal neurons on adjacent slides using NeuN immunostaining in order to evaluate the relationship between neuronal density, total 5-HT_{1A} receptors, and functional receptors. In the CA1 area, we only found a significant decrease of neuronal density in the Braak V/VI group, although there was a downward trend in the Braak I/II group. In the dentate gyrus, the neuronal density was significantly increased in the Braak I/II group and unchanged in the other groups. Interestingly, in both regions, these longitudinal modifications paralleled those observed with [^{18}F]MPPF binding. In agreement with previous findings (Kepe et al., 2006; Mizukami et al., 2011), our observations suggest that the total density of 5-HT_{1A} receptors in the hippocampus (regardless of their coupling state) is related to the neuronal density. Interestingly, in the CA1 hippocampal subfield, [^{18}F]F13640 binding clearly appeared to be decreased earlier and to a greater extent than the neuronal loss. The functional impairment of 5-HT_{1A} receptors might therefore be related to the presence of neurofibrillary tangles rather than to decreases in neuronal density.

Finally, the results of this in vitro study have a strong translational transferability. We demonstrated the hypothesis that agonist and antagonist radiotracers bind differently to 5-HT_{1A} receptors in human brain tissue, revealing distinct GPCR coupling states. The extent of 5-HT_{1A} receptor G-protein coupling may provide original information to understand mechanisms underlying neurotransmission impairment during Alzheimer's disease. We chose fluorinated 5-HT_{1A} receptors radioligands which are directly transferable to PET imaging *in vivo*: the antagonist [^{18}F]MPPF is a widely-used PET radiopharmaceutical and the agonist radiotracer we developed, [^{18}F]F13640, will soon be available for human studies because its first-in-man study is in progress (EU Clinical Trials

Register, EudraCT number #2016-00016-15). The present results therefore support a new neuropharmacological concept for nuclear medicine consisting in comparing the binding profiles of a PET agonist and an antagonist in subjects to determine in vivo the extent of G-protein coupling of 5-HT_{1A} receptors and to follow its modifications in functionality during the neurodegenerative process. More widely, this concept could also be explored in other neuropsychiatric diseases involving changes in 5-HT_{1A} receptor expression and function and in the exploration of their respective therapeutics.

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Dr. Newman-Tancredi is an employee and stockholder of Neurolixis but has no financial disclosures associated with this project. The other authors report no conflict of interest and have nothing to disclose.

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References

Alafuzoff, I., Arzberger, T., Al-Sarraj, S., Bodi, I., Bogdanovic, N., Braak, H., Bugiani, O., Del-Tredici, K., Ferrer, I., Gelpi, E., Giaccone, G., Graeber, M. B., Ince, P., Kamphorst, W., King, A., Korkolopoulou, P., Kovacs, G. G., Larionov, S., Meyronet, D., Monoranu, C., Parchi, P., Patsouris, E., Roggendorf, W., Seilhean, D., Tagliavini, F., Stadelmann, C., Streichenberger, N., Thal, D. R., Wharton, S. B., Kretzschmar, H., 2008. Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. Brain Pathol 18, 484-496.

Albert, P. R., Vahid-Ansari, F., Luckhart, C., 2014. Serotonin-prefrontal cortical circuitry in anxiety and depression phenotypes: pivotal role of pre- and post-synaptic 5-HT1A receptor expression. Front Behav Neurosci 8, 199.

Aznavour, N., Zimmer, L., 2007. [18F]MPPF as a tool for the in vivo imaging of 5-HT1A receptors in animal and human brain. Neuropharmacology 52, 695-707.

Bakker, A., Krauss, G. L., Albert, M. S., Speck, C. L., Jones, L. R., Stark, C. E., Yassa, M. A., Bassett, S. S., Shelton, A. L., Gallagher, M., 2012. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron 74, 467-474.

Becker, G., Streichenberger, N., Billard, T., Newman-Tancredi, A., Zimmer, L., 2014. A postmortem study to compare agonist and antagonist 5-HT1A receptor-binding sites in Alzheimer's disease. CNS Neurosci Ther 20, 930-934.

Boekhoorn, K., Joels, M., Lucassen, P. J., 2006. Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. Neurobiol Dis 24, 1-14.

Borg, J., 2008. Molecular imaging of the 5-HT1A receptor in relation to human cognition. Behav Brain Res 195, 103-111.

Bowen, D. M., Najlerahim, A., Procter, A. W., Francis, P. T., Murphy, E., 1989. Circumscribed changes of the cerebral cortex in neuropsychiatric disorders of later life. Proc Natl Acad Sci U S A 86, 9504-9508.

Burnet, P. W., Eastwood, S. L., Harrison, P. J., 1997. [3H]WAY-100635 for 5-HT1A receptor autoradiography in human brain: a comparison with [3H]8-OH-DPAT and demonstration of increased binding in the frontal cortex in schizophrenia. Neurochem Int 30, 565-574.

Cheng, K.T., 2005. 4-(2´-Methoxyphenyl)-1-[2´-(N-2´´-pyridinyl)-p-[18F]fluorobenzamido]ethylpiperazine. Molecular Imaging and Contrast Agent Database (MICAD). Bethesda (MD): National Center for Biotechnology Information (US).

Colpaert, F. C., Tarayre, J. P., Koek, W., Pauwels, P. J., Bardin, L., Xu, X. J., Wiesenfeld-Hallin, Z., Cosi, C., Carilla-Durand, E., Assie, M. B., Vacher, B., 2002. Large-amplitude 5-HT1A receptor activation: a new mechanism of profound, central analgesia. Neuropharmacology 43, 945-958.

Cross, A. J., Slater, P., Perry, E. K., Perry, R. H., 1988. An autoradiographic analysis of serotonin receptors in human temporal cortex: Changes in Alzheimer-type dementia. Neurochem Int 13, 89-96.

de Flores, R., La Joie, R., Chetelat, G., 2015. Structural imaging of hippocampal subfields in healthy aging and Alzheimer's disease. Neuroscience 309, 29-50.

DeKosky, S. T., Ikonomovic, M. D., Styren, S. D., Beckett, L., Wisniewski, S., Bennett, D. A., Cochran, E. J., Kordower, J. H., Mufson, E. J., 2002. Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. Ann Neurol 51, 145-155.

Depoortere, R., Auclair, A. L., Bardin, L., Colpaert, F. C., Vacher, B., Newman-Tancredi, A., 2010. F15599, a preferential post-synaptic 5-HT1A receptor agonist: activity in models of cognition in comparison with reference 5-HT1A receptor agonists. Eur Neuropsychopharmacol 20, 641-654.

Dickerson, B. C., Salat, D. H., Bates, J. F., Atiya, M., Killiany, R. J., Greve, D. N., Dale, A. M., Stern, C. E., Blacker, D., Albert, M. S., Sperling, R. A., 2004. Medial temporal lobe function and structure in mild cognitive impairment. Ann Neurol 56, 27-35.

Dickerson, B. C., Salat, D. H., Greve, D. N., Chua, E. F., Rand-Giovannetti, E., Rentz, D. M., Bertram, L., Mullin, K., Tanzi, R. E., Blacker, D., Albert, M. S., Sperling, R. A., 2005. Increased hippocampal activation in mild cognitive impairment compared to normal aging and AD. Neurology 65, 404-411.

Donovan, M. H., Yazdani, U., Norris, R. D., Games, D., German, D. C., Eisch, A. J., 2006. Decreased adult hippocampal neurogenesis in the PDAPP mouse model of Alzheimer's disease. J Comp Neurol 495, 70-83.

Dubois, B., Feldman, H. H., Jacova, C., Dekosky, S. T., Barberger-Gateau, P., Cummings, J., Delacourte, A., Galasko, D., Gauthier, S., Jicha, G., Meguro, K., O'Brien, J., Pasquier, F., Robert, P., Rossor, M., Salloway, S., Stern, Y., Visser, P. J., Scheltens, P., 2007. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 6, 734-746.

Duvernoy, H.M., Cattin, F., Fatterpekar, G., Naidich, T., Raybaud, C., Risold P.Y., Salvolini, U., Scarabino, T., 2005. The Human Hippocampus, Third ed., Springer.

Garcia-Jimenez, A., Cowburn, R. F., Ohm, T. G., Lasn, H., Winblad, B., Bogdanovic, N., Fastbom, J., 2002. Loss of stimulatory effect of guanosine triphosphate on [(35)S]GTPgammaS binding correlates with Alzheimer's disease neurofibrillary pathology in entorhinal cortex and CA1 hippocampal subfield. J Neurosci Res 67, 388-398.

Gonzalez-Maeso, J., Torre, I., Rodriguez-Puertas, R., Garcia-Sevilla, J. A., Guimon, J., Meana, J. J., 2002. Effects of age, postmortem delay and storage time on receptor-mediated activation of G-proteins in human brain. Neuropsychopharmacology 26, 468-478.

Gozlan, H., Thibault, S., Laporte, A. M., Lima, L., Hamon, M., 1995. The selective 5-HT1A antagonist radioligand [3H]WAY 100635 labels both G-protein-coupled and free 5-HT1A receptors in rat brain membranes. Eur J Pharmacol 288, 173-186.

Halliday, G. M., McCann, H. L., Pamphlett, R., Brooks, W. S., Creasey, H., McCusker, E., Cotton, R. G., Broe, G. A., Harper, C. G., 1992. Brain stem serotonin-synthesizing neurons in Alzheimer's disease: a clinicopathological correlation. Acta Neuropathol 84, 638-650.

Heusler, P., Palmier, C., Tardif, S., Bernois, S., Colpaert, F. C., Cussac, D., 2010. [(3)H]-F13640, a novel, selective and high-efficacy serotonin 5-HT(1A) receptor agonist radioligand. Naunyn Schmiedebergs Arch Pharmacol 382, 321-330.

Hirst, W. D., Cheung, N. Y., Rattray, M., Price, G. W., Wilkin, G. P., 1998. Cultured astrocytes express messenger RNA for multiple serotonin receptor subtypes, without functional coupling of 5-HT1 receptor subtypes to adenylyl cyclase. Brain Res Mol Brain Res 61, 90-99.

Jin, K., Peel, A. L., Mao, X. O., Xie, L., Cottrell, B. A., Henshall, D. C., Greenberg, D. A., 2004. Increased hippocampal neurogenesis in Alzheimer's disease. Proc Natl Acad Sci U S A 101, 343-347.

Kepe, V., Barrio, J. R., Huang, S. C., Ercoli, L., Siddarth, P., Shoghi-Jadid, K., Cole, G. M., Satyamurthy, N., Cummings, J. L., Small, G. W., Phelps, M. E., 2006. Serotonin 1A receptors in the living brain of Alzheimer's disease patients. Proc Natl Acad Sci U S A 103, 702-707.

Kia, H. K., Miquel, M. C., Brisorgueil, M. J., Daval, G., Riad, M., El Mestikawy, S., Hamon, M., Verge, D., 1996. Immunocytochemical localization of serotonin1A receptors in the rat central nervous system. J Comp Neurol 365, 289-305.

Kung, H. F., Stevenson, D. A., Zhuang, Z. P., Kung, M. P., Frederick, D., Hurt, S. D., 1996. New 5-HT1A receptor antagonist: [3H]p-MPPF. Synapse 23, 344-346.

Lanctot, K. L., Hussey, D. F., Herrmann, N., Black, S. E., Rusjan, P. M., Wilson, A. A., Houle, S., Kozloff, N., Verhoeff, N. P., Kapur, S., 2007. A positron emission tomography study of 5-hydroxytryptamine-1A receptors in Alzheimer disease. Am J Geriatr Psychiatry 15, 888-898.

Le Bars, D., Lemaire, C., Ginovart, N., Plenevaux, A., Aerts, J., Brihaye, C., Hassoun, W., Leviel, V., Mekhsian, P., Weissmann, D., Pujol, J. F., Luxen, A., Comar, D., 1998. High-yield radiosynthesis and preliminary in vivo evaluation of p-[18F]MPPF, a fluoro analog of WAY-100635. Nucl Med Biol 25, 343-350.

Lemoine, L., Verdurand, M., Vacher, B., Blanc, E., Le Bars, D., Newman-Tancredi, A., Zimmer, L., 2010. [18F]F15599, a novel 5-HT1A receptor agonist, as a radioligand for PET neuroimaging. Eur J Nucl Med Mol Imaging 37(3), 594-605.

Lorke, D. E., Lu, G., Cho, E., Yew, D. T., 2006. Serotonin 5-HT2A and 5-HT6 receptors in the prefrontal cortex of Alzheimer and normal aging patients. BMC Neurosci 7, 36.

Mai, J.K., Paxinos, G., 1997. Atlas of the human brain. CA: Academic Press, San Diego.

Maurel, J. L., Autin, J. M., Funes, P., Newman-Tancredi, A., Colpaert, F., Vacher, B., 2007. High-efficacy 5-HT1A agonists for antidepressant treatment: a renewed opportunity. J Med Chem 50, 5024-5033.

Middlemiss, D. N., Palmer, A. M., Edel, N., Bowen, D. M., 1986. Binding of the novel serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin in normal and Alzheimer brain. J Neurochem 46, 993-996.

Mizukami, K., Ishikawa, M., Akatsu, H., Abrahamson, E. E., Ikonomovic, M. D., Asada, T., 2011. An immunohistochemical study of the serotonin 1A receptor in the hippocampus of subjects with Alzheimer's disease. Neuropathology 31, 503-509.

Mongeau, R., Welner, S. A., Quirion, R., Suranyi-Cadotte, B. E., 1992. Further evidence for differential affinity states of the serotonin1A receptor in rat hippocampus. Brain Res 590, 229-238.

Mu, Y., Gage, F. H., 2011. Adult hippocampal neurogenesis and its role in Alzheimer's disease. Mol Neurodegener 6, 85.

Nenonene, E. K., Radja, F., Carli, M., Grondin, L., Reader, T. A., 1994. Heterogeneity of cortical and hippocampal 5-HT1A receptors: a reappraisal of homogenate binding with 8-[3H]hydroxydipropylaminotetralin. J Neurochem 62, 1822-1834.

Polter, A. M., Li, X., 2010. 5-HT1A receptor-regulated signal transduction pathways in brain. Cell Signal 22, 1406-1412.

Ramirez, M. J., Lai, M. K., Tordera, R. M., Francis, P. T., 2014. Serotonergic therapies for cognitive symptoms in Alzheimer's disease: rationale and current status. Drugs 74, 729-736.

Raskin, J., Cummings, J., Hardy, J., Schuh, K., Dean, R. A., 2015. Neurobiology of Alzheimer's Disease: Integrated Molecular, Physiological, Anatomical, Biomarker, and Cognitive Dimensions. Curr Alzheimer Res 12, 712-722.

Salmon, E., 2007. A review of the literature on neuroimaging of serotoninergic function in Alzheimer's disease and related disorders. J Neural Transm (Vienna) 114, 1179-1185.

Sarnyai, Z., Sibille, E. L., Pavlides, C., Fenster, R. J., McEwen, B. S., Toth, M., 2000. Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin(1A) receptors. Proc Natl Acad Sci U S A 97, 14731-14736.

Sato, S., Mizukami, K., Asada, T., 2007. A preliminary open-label study of 5-HT1A partial agonist tandospirone for behavioural and psychological symptoms associated with dementia. Int J Neuropsychopharmacol 10, 281-283.

Schechter, L. E., Smith, D. L., Rosenzweig-Lipson, S., Sukoff, S. J., Dawson, L. A., Marquis, K., Jones, D., Piesla, M., Andree, T., Nawoschik, S., Harder, J. A., Womack, M. D., Buccafusco, J., Terry, A. V., Hoebel, B., Rada, P., Kelly, M., Abou-Gharbia, M., Barrett, J. E., Childers, W., 2005. Lecozotan (SRA-333): a selective serotonin 1A receptor antagonist that enhances the stimulated release of glutamate and acetylcholine in the hippocampus and possesses cognitive-enhancing properties. J Pharmacol Exp Ther 314, 1274-1289.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9, 671-675.

Serrano-Pozo, A., Frosch, M. P., Masliah, E., Hyman, B. T., 2011. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 1, a006189.

Tahmasian, M., Pasquini, L., Scherr, M., Meng, C., Forster, S., Mulej Bratec, S., Shi, K., Yakushev, I., Schwaiger, M., Grimmer, T., Diehl-Schmid, J., Riedl, V., Sorg, C., Drzezga, A., 2015. The lower hippocampus global connectivity, the higher its local metabolism in Alzheimer disease. Neurology 84, 1956-1963.

Tampellini, D., 2015. Synaptic activity and Alzheimer's disease: a critical update. Front Neurosci 9, 423.

Thathiah, A., De Strooper, B., 2011. The role of G protein-coupled receptors in the pathology of Alzheimer's disease. Nat Rev Neurosci 12, 73-87.

Truchot, L., Costes, N., Zimmer, L., Laurent, B., Le Bars, D., Thomas-Anterion, C., Mercier, B., Hermier, M., Vighetto, A., Krolak-Salmon, P., 2008. A distinct [18F]MPPF PET profile in amnestic mild cognitive impairment compared to mild Alzheimer's disease. Neuroimage 40, 1251-1256.

Truchot, L., Costes, S. N., Zimmer, L., Laurent, B., Le Bars, D., Thomas-Anterion, C., Croisile, B., Mercier, B., Hermier, M., Vighetto, A., Krolak-Salmon, P., 2007. Up-regulation of hippocampal serotonin metabolism in mild cognitive impairment. Neurology 69, 1012-1017.

Verdurand, M., Berod, A., Le Bars, D., Zimmer, L., 2011. Effects of amyloid-beta peptides on the serotoninergic 5-HT1A receptors in the rat hippocampus. Neurobiol Aging 32, 103-114.

Verdurand, M., Chauveau, F., Daoust, A., Morel, A.L., Bonnefoi, F., Liger, F., Berod, A., Zimmer, L., 2016. Neurobiol Aging 40, 11-21. In press.

Vidal, B., Fieux, S.; Billard, T., Newman-Tancredi, A., Zimmer, L., 2014. Radiopharmacological evaluation of [18F]F13640, a novel 5-HT1A receptor agonist. J Nuclear Medicine 55, 1100.

Weinstein, D., Magnuson, D., Lee, J., 1996. Altered G-protein coupling of a frontal cortical low affinity [3H]8-hydroxy-N,N-dipropyl-2-aminotetralin serotonergic binding site in Alzheimer's disease. Behav Brain Res 73, 325-329.

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Table 1. Demographic data of the patients

Braak stages	Control	I/II	III/IV	V/VI
Number of cases	9	5	10	10
Age (years)	79.3 ± 12.1	84.2 ± 8.8	83.5 ± 6.9	75.5 ± 12.0
Gender (M/F)	6/3	2/3	5/5	7/3
Postmortem interval (h)	35.8 ± 12.7	36.1 ± 10.5	35.2 ± 18.1	41.4 ± 23.0

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Figure 1. Regional distribution of [^{18}F]F13640 and [^{18}F]MPPF binding sites in the hippocampus of a control subject (DG, dentate gyrus; CA1, CA1 area on the corresponding anatomic slice modified from Duvernoy et al., 2005).

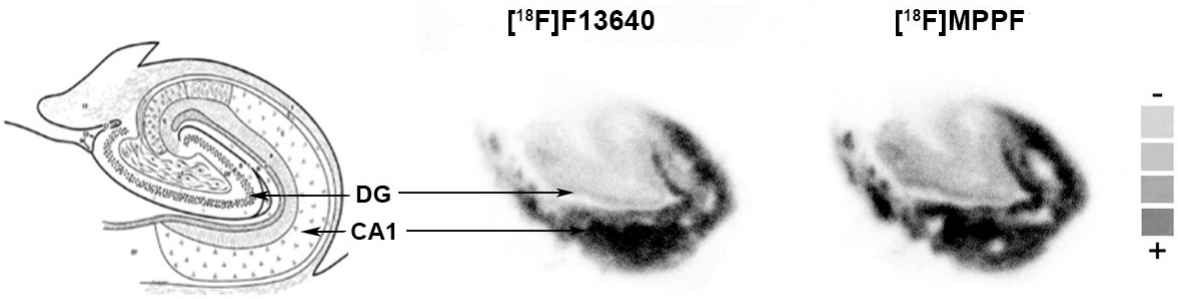

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Figure 2. Regional density of NeuN in the hippocampus of control subject (entire image obtained by a slide scanner; same patient as shown in Figure 1). Boxes highlight areas where neuronal densities were measured: (A), CA1 area; (B) dentate gyrus area.

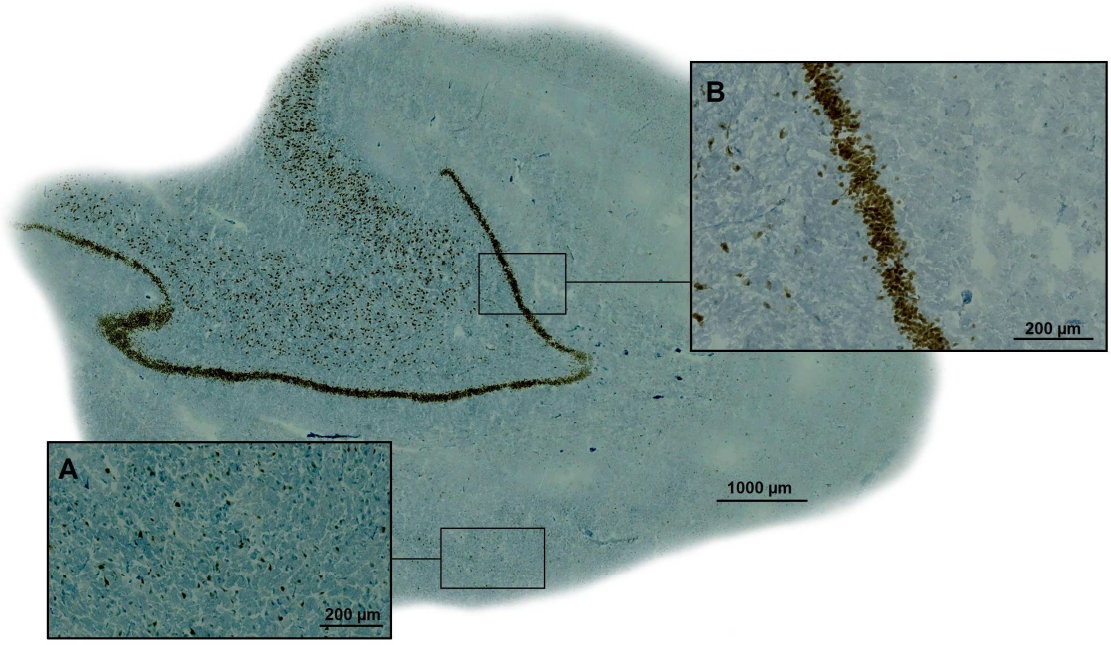

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Figure 3. Differential effects of Gpp(NH)p, decoupling receptors from G proteins, on the two 5-HT_{1A} receptors radiotracers. The antagonist [^{18}F]MPPF binds to all 5-HT_{1A} receptors, both in a G-protein coupled receptor state and uncoupled state. The agonist [^{18}F]F13640 binds mostly to the G-protein coupled receptor state (functional receptors). (A) Autoradiographs showing the decrease of [^{18}F]F13640 binding when Gpp(NH)p is added during incubation, both in a control subject (left slices) and in an AD subject (right slices). (B) Autoradiographs showing the light increase of [^{18}F]MPPF binding when Gpp(NH)p is added during incubation, both in a control subject (left slices) and in an AD subject (right slices). (C) Quantification of [^{18}F]F13640 binding in the hippocampus after Gpp(NH)p decoupling (n = 18 subjects) : -59% in the dentate gyrus (DG) and -75% in CA1 area (CA1), both at $p<0.001$ with one-sample t-test. (D) Quantification of [^{18}F]MPPF binding in the hippocampus after Gpp(NH)p decoupling (n = 16 subjects) : +17% in the dentate gyrus ($p<0.01$) and + 18% in CA1 ($p<0.001$), one-sample t-test.

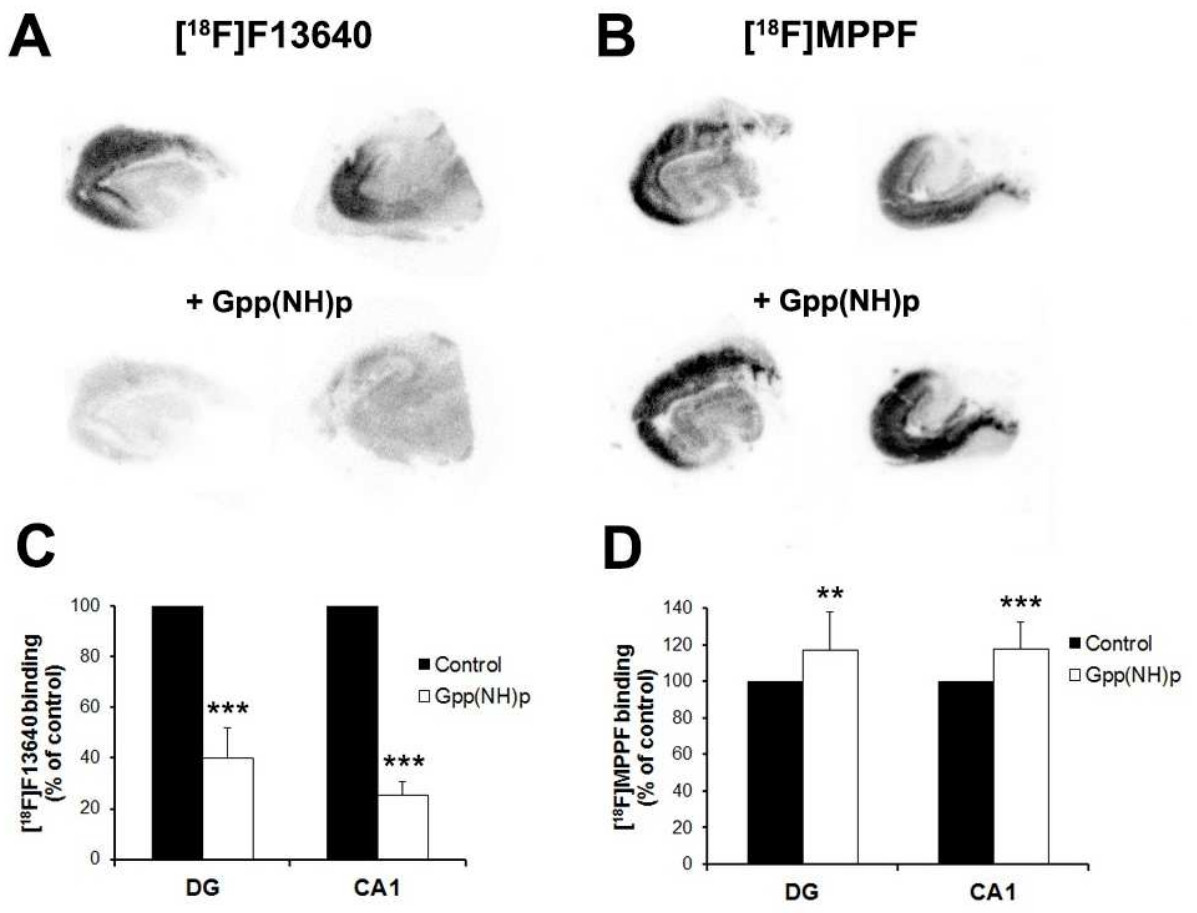
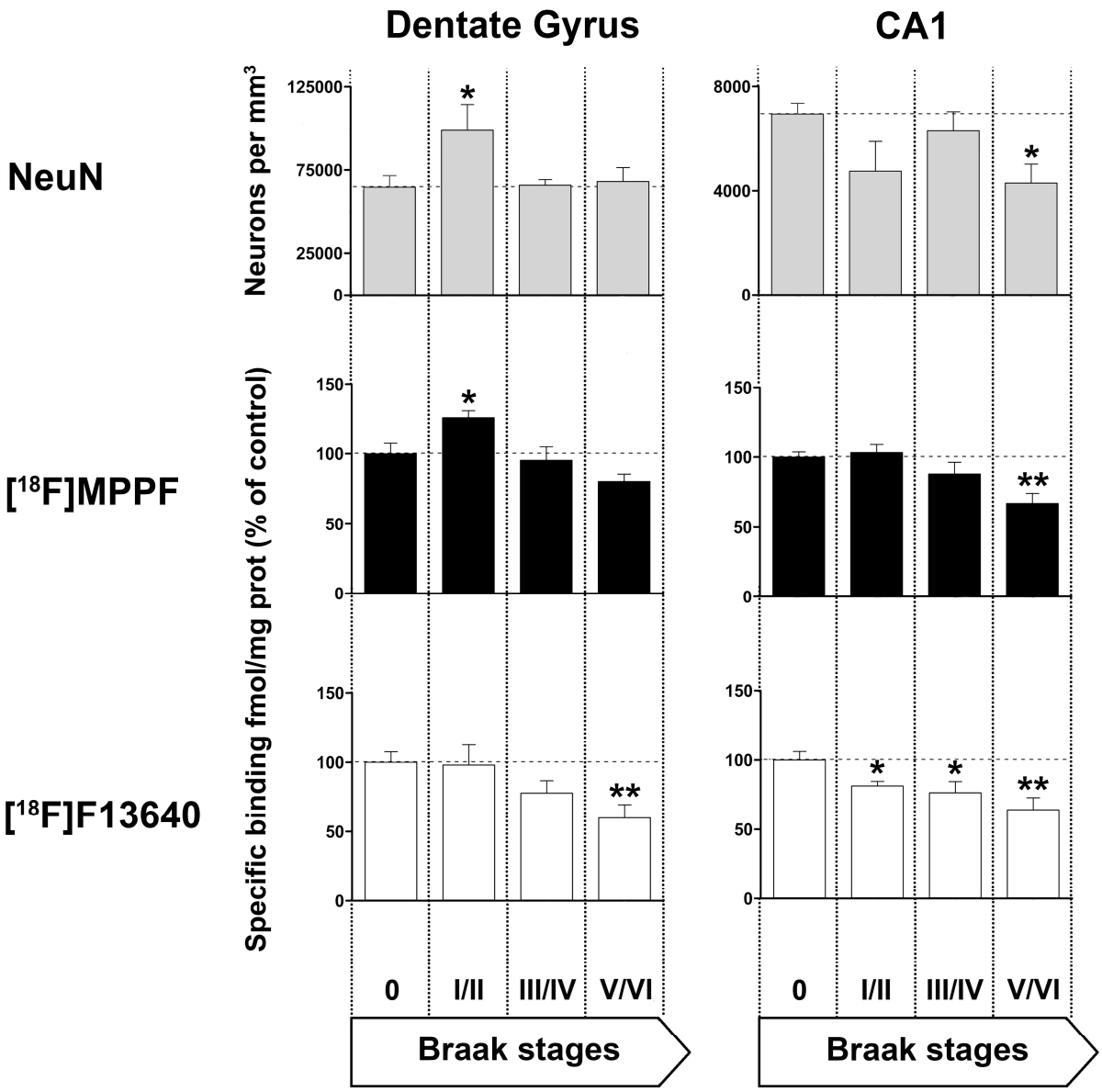


Figure 4. Neuronal density (as revealed by NeuN immunostaining) and 5-HT_{1A} receptor binding site densities (according to the [^{18}F]MPPF and [^{18}F]F13640 binding in the dentate gyrus and CA1 areas of Alzheimer's disease patients at different Braak stages. Results are expressed as a percentage of the control group (Braak stage 0). *p<0.05; **p<0.01, Mann-Whitney nonparametric t-test.



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Highlights

- 5-HT_{1A} receptor autoradiography was performed in hippocampi of AD patients.
- Two 5-HT_{1A} PET ligands were compared: an agonist, [^{18}F]F13640, *vs* an antagonist, [^{18}F]MPPF.
- [^{18}F]F13640 labels specifically G-coupled receptors, i.e. the functional receptors.
- [^{18}F]MPPF labels all 5-HT_{1A} receptors, regardless of their G-protein coupling state.
- Comparison of these distinct bindings is proposed as new neuropharmacological paradigm for PET.

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